

Amendments to the specification

At the indicated page and line numbers, please replace the existing paragraphs with those set forth below:

(Page 10, line 1 through page 13, line 29)

Referring to ~~fig.~~Fig. 1 a syringe (1) having a MOVEABLE piston (2) has an adsorbent solid phase (3) held within it. In use the nozzle (4) is placed within the liquid from which material is to be separated and the piston withdrawn to suck up the liquid through (3). When the piston is depressed the liquid is forced back over (3) and this process can be repeated if desired so that there is better adsorption of material from the liquid.

Referring to ~~fig.~~Fig. 2 the syringe (5) with a piston (6) has nozzle (7) placed in cartridge (8) containing a solid adsorbent and the cartridge (8) has its inlet (9) placed in the liquid from which material is to be separated. When the piston (6) is withdrawn the liquid is drawn up through the cartridge and material is adsorbed, when the piston is depressed the liquid is forced back over the adsorbent in the cartridge so that there is better adsorption of material from the liquid.

Referring to ~~fig.~~Fig. 4 the adsorbent material (10) can be in the form of frits or beads and can fill the cartridge.

Referring to ~~fig.~~Fig. 5 there can be a by-pass channel (11) round the outside of the solid adsorbent so that larger particles can pass up and down without clogging.

Referring to ~~fig.~~Fig. 6 there are discs (12) positioned within the cartridge and each disc consists of an adsorbent membrane, the discs can have large pores as illustrated in ~~fig.~~Fig. 7 and can have cut away sections as shown in ~~fig.~~Fig. 8 to prevent blocking. The discs can be stacked on top the other and can have a raised lip (14) as shown in ~~fig.~~Fig. 9 so that the discs are

only in contact through this lip.

Referring to ~~fig.~~Fig. 3 a pipette (15) has an aerosol plug (16) to prevent contamination and contains a plug ~~(16)~~(18) of adsorbent material such as a porous plastic material as shown. In use the tip of the pipette (17) is placed in the liquid and liquid is sucked up over the plug ~~(16)~~(18), by blowing down the pipette the liquid is forced back over the plug ~~(16)~~(18) so that there is better adsorption of material from the liquid.

The adsorbed material can be removed from the solid phase by conventional elution methods.

The invention is described in the following examples in which the isolated or eluted products were identified using conventional laboratory analysis methods.

Example 1

Using the equipment of ~~fig.~~Fig. 2 polystyrene porous carboxylated beads (200-500 microns or 16-50 mesh size) were loaded into a chromatography cartridge and held in place with plastic mesh with pore sizes of about 100 microns.

Whole blood was diluted 10 times with 10mM Ammonium Bicarbonate, 10mM Ammonium Carbonate and 0.1 % ~~Tween~~TWEEN 20 (poly(oxyethylene) (20)-sorbitan monolaureate) pH9 and sucked up and down the cartridge with a syringe and passed back through the cartridge. The dilution buffer can be any hypotonic solution that causes lysis of the red blood cell fraction, but maintains the integrity of nuclei, white blood cells or chromatin. The nuclei became immobilized on the beads and the lysed blood was taken to waste. Direct elution of the nuclear DNA was achieved using hot water. To obtain greater purity DNA, the eluate from the first cartridge was then further processed using another cartridge containing a solid-phase with poly imidazole groups.

To collect the white blood cell fraction, the same solution is made isotonic with saline and the cells were captured in a similar manner.

Example 2

Using the equipment of ~~fig.~~Fig. 3 polystyrene porous carboxylated beads (200-500 microns or 16-50 mesh size) were loaded into a 1 ml pipette tip.

Whole blood was diluted 10 times with 10mM Ammonium Bicarbonate and 0.01% ~~Tween~~TWEEN 20 (poly(oxyethylene) (20)-sorbitan monolaureate) pH9 and sucked up and down the tip of the pipette. The nuclei became immobilized on the beads and the lysed blood was removed to waste. Direct elution of the nuclear DNA was achieved using alkaline detergent solutions and by boiling water.

Example 3

Using the equipment of ~~fig.~~Fig. 2 agarose was treated with Carbonyldiimidazole in anhydrous organic solvent and then left in water at pH 3 to maintain the imidazole groups. The derivatised agarose was placed in a cartridge and the supernatant from a plasmid alkaline lysis preparation was sucked up and down immobilizing the plasmid DNA on the beads at pH 5. After washing, the plasmid DNA was eluted with 1 0mM Tris HCl, pH 9.

The above was repeated with carboxylated polystyrene and dextrans of various sizes and DNA obtained by elution as above.

Example 4

Extraction of nuclei or DNA from whole blood

Using the equipment of ~~fig.~~Fig. 2 with the packing of ~~fig.~~Fig. 6 whole blood was lysed with 5 volumes of 10mM Ammonium Bicarbonate containing 0.1 % ~~Tween~~TWEEN 20 (poly(oxyethylene)

(20)-sorbitan monolaureate) pH9. The lysed blood was passed through several 20micron porous polyethylene frits modified with larger pores of 1 mm in diameter, housed in a plastic cartridge attached to a 2ml syringe and plunger. Each frit was spaced 3mm apart to allow free flow of liquid.

The nuclei or white blood cell fraction bound to the frit allowing all the contaminating proteins and lipids to pass through to waste in a single pass or several strokes of the plunger. The frit and nuclei was then washed to remove residual proteins using deionised water or chaotropes or alcohols or detergents such SDS or ~~Tween~~TWEEN 20 (poly(oxyethylene) (20)-sorbitan monolaureate) or combinations or lactic and salicylic acids or their salts, or poly phosphates or per chlorates and either eluted off using hot water or alkaline solutions of detergents or further purified inside the cartridge using chaotropic agents or proteases.

Example 5

Purification of buccal cell DNA

Using the equipment of ~~fig.~~Fig. 3 a plug of porous polyethylene was derivatised with imidazole groups and inserted into the tip of a standard IMI pipette tip. A further non derivatised plug was inserted at the top to act as an aerosol and liquid barrier to prevent contamination of the pipette.

A buccal scrape was taken and mixed with 0.2M guanidine isothiocyanate, 3% ~~Tween~~TWEEN 20 (poly(oxyethylene) (20)-sorbitan monolaureate), Proteinase K and 50mM MES pH5 at 30°C for 15 minutes. The mixture was then sucked up and down the tip several times allowing the DNA to bind to the derivatised plug. The plug was washed with 1mM MES pH5 and then the DNA eluted with 10mM Tris. HCl pH9. The same protocol was repeated using 0.01% to 10% SDS with or without salts and buffers. Fast degradation of the buccal cells can also be achieved using salicylic acid, lactic

acid, or MgCl₂ at concentrations of 0.05 to 5M. Combinations of the above salts and reagents can also be used.

Example 6

1 gram of carboxylated polystyrene beads with a diameter of about 60 microns or 200 to 400 mesh was suspended in a hypotonic solution of ammonium bicarbonate 1 OmM with 0.1 % ~~Tween~~TWEEN 20 (poly(oxyethylene) (20)-sorbitan monolaureate) pH9. A five fold excess of this suspension was added to a 5ml blood sample and mixed once. The beads captured the nuclei and sedimented. After several washes with water, the DNA was eluted with hot water. To concentrate the DNA the equipment of ~~fig.~~Fig. 2 was used with the packing of ~~fig.~~Fig. 6 and the DNA was captured on a porous disc in the cartridge and subsequently eluted off in a small volume and analysed using PCR or Restriction Digestion.

Example 7

Removal and purification of human IgG from serum

An agarose gel coupled to Protein A was placed in the cartridge of ~~fig.~~Fig. 2 and washed with phosphate buffered saline. A solution containing human IgG in serum was sucked up and down the solid phase until all the IgG was bound. After washing the solid phase with PBS, the IgG was eluted with 0.1 M glycine, 0.1 5M NaCl, pH2.8 and immediately neutralised with Tris. HCl.

(Page 14, line 16 through page 15, line 3)

Example 10

Extraction of HIV RNA from serum

A cartridge as in ~~fig.~~Fig. 2 was packed with 60 micron silica and a sample of serum diluted 5 times with 6M guanidine isothiocyanate, 0.1 % TWEEN 20 (poly(oxyethylene) (20)-sorbitan monolaureate), 20mM EDTA, 100mM Tris. HCl pH6 was sucked up and

down through the solid phase. After washing the solid phase with isopropanol and drying the RNA was eluted using water at 60C.

Example 11

Purification of PCR reactions

A cartridge as in ~~fig.~~Fig. 2 was packed with 60 micron silica and a sample of a PCR reaction diluted 5 times with 6M guanidine isothiocyanate, 0.1 % TWEEN 20 (poly(oxyethylene) (20)-sorbitan monolaureate), 20mM EDTA, 100mM TRIS-HCL pH6 was sucked up through the solid phase. After washing the solid phase with isopropanol and drying the DNA was eluted using water.

Example 12

Extraction of RNA from Liver

Fresh liver was homogenized in a mixture of 50% Phenol containing 6M Guanidine isothiocyanate, 10mM DTT, 0.1 M Sodium Acetate pH 4. Chloroform was added to separate the phases and the top layer containing the RNA was sucked up and down through a cartridge of ~~fig.~~Fig. 2 containing 60micron silica. The silica was washed with alcohol, air dried and the RNA eluted with hot water ready for processing.

(Page 15, line 24 through page 16, line 2)

Example 15

Use of electrodes, static charge, induction, electrophoresis to isolate DNA or RNA

Whole blood was diluted down 10 times in 1 OmM ammonium carbonate/bicarbonate, 50mM Tris. HCl with 1 % TWEEN 20 (poly(oxyethylene) (20)-sorbitan monolaureate), 1001g/ml proteinase K pH9. Electrodes were surrounded by dialysis tubing containing the same buffer and dipped into the solution. A 12

volt direct current from a battery was connected and the nuclei or DNA was captured on the outside of the dialysis tubing at the positive electrode after a 1 hour incubation. The DNA could be removed by elution with water.